Journal of Autoimmunity xxx (2015) 1–8

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Genome wide identification of new genes and pathways in patients with both autoimmune thyroiditis and type 1 diabetes

Yaron Tomer [a, b, *], Lawrence M. Dolan [c], George Kahaly [d], Jasmin Divers [e], Ralph B. D'Agostino Jr. [e], Giuseppina Imperatore [f], Dana Dabelea [g], Santica Marcovina [h], Mary Helen Black [i], Catherine Pihoker [j], Alia Hasham [a], Sara Salehi Hammerstad [a], David A. Greenberg [k], Vaneet Lotay [l], Weijia Zhang [l], Maria Cristina Monti [m], Nina Matheis [d], on behalf of the SEARCH for Diabetes in Youth Study

[a] Division of Endocrinology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[b] James J. Peters Veterans Affairs Medical Center, Bronx, NY, USA
[c] Division of Pediatric Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[d] Thyroid Research Laboratory, Johannes Gutenberg University Medical Center, Mainz, Germany
[e] Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, NC, USA
[f] Division of Diabetes Translation, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA
[g] Colorado School of Public Health, University of Colorado, Denver, CO, USA
[h] Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington, Seattle, WA, USA
[i] Kaiser Permanente Southern California, Department of Research and Evaluation, Pasadena, CA, USA
[j] Department of Pediatrics, University of Washington, Seattle, WA, USA
[k] Battelle Center for Mathematical Medicine, Nationwide Children's Hospital, Columbus, OH, USA
[l] The Charles Bronfman Institute for Personalized Medicine, Department of Medicine Bioinformatics Core, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[m] Department of Public Health, Neurosciences, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form
27 March 2015
Accepted 29 March 2015
Available online xxx

Keywords:
Type 1 diabetes
Graves' disease
Hashimoto's thyroiditis
Gene
HLA

ABSTRACT

Autoimmune thyroid diseases (AITD) and Type 1 diabetes (T1D) frequently occur in the same individual pointing to a strong shared genetic susceptibility. Indeed, the co-occurrence of T1D and AITD in the same individual is classified as a variant of the autoimmune polyglandular syndrome type 3 (designated APS3v). Our aim was to identify new genes and mechanisms causing the co-occurrence of T1D + AITD (APS3v) in the same individual using a genome-wide approach. For our discovery set we analyzed 346 Caucasian APS3v patients and 727 gender and ethnicity matched healthy controls. Genotyping was performed using the Illumina Human660W-Quad.v1. The replication set included 185 APS3v patients and 340 controls. Association analyses were performed using the PLINK program, and pathway analyses were performed using the MAGENTA software. We identified multiple signals within the HLA region and conditioning studies suggested that a few of them contributed independently to the strong association of the HLA locus with APS3v. Outside the HLA region, variants in GPR103, a gene not suggested by previous studies of APS3v, T1D, or AITD, showed genome-wide significance ($p < 5 \times 10^{-8}$). In addition, a locus on 1p13 containing the PTPN22 gene showed genome-wide significant associations. Pathway analysis demonstrated that cell cycle, B-cell development, CD40, and CTLA-4 signaling were the major pathways contributing to the pathogenesis of APS3v. These findings suggest that complex mechanisms involving T-cell and B-cell pathways are involved in the strong genetic association between AITD and T1D.

Published by Elsevier Ltd.

1. Introduction

The most common autoimmune endocrine disorders are type 1 (autoimmune) diabetes (T1D) and autoimmune thyroid diseases

* Corresponding author. Division of Endocrinology, Box 1055, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029, USA. Tel.: +1 212 241 1877; fax: +1 212 241 4218.
E-mail address: Yaron.Tomer@mssm.edu (Y. Tomer).

http://dx.doi.org/10.1016/j.jaut.2015.03.006
0896-8411/Published by Elsevier Ltd.

(AITD). T1D and AITD are both characterized by T-cell infiltration and production of autoantibodies directed at the target organs (pancreatic islets and thyroid, respectively), resulting in their dysfunction or destruction [1]. Epidemiological data have shown that T1D and AITD frequently occur together in the same family and in the same individual, suggesting a strong shared genetic susceptibility [1]. In different studies, up to 44% of T1D patients were positive for thyroid antibodies (TAb) (thyroid peroxidase [TPO] and/or thyroglobulin [Tg] antibodies) [2,3]. Similarly, 2.3% of children with AITD have islet cell antibodies compared with 0% of controls [4]. Indeed, the co-occurrence of T1D and AITD in the same individual is classified as one of the variants of autoimmune polyglandular syndrome type 3 (APS3) [5] (since the phenotype of T1D + AITD in the same individual is a known as a variant of APS3 we refer to it as APS3v in this manuscript).

Family studies also support a strong shared genetic susceptibility to T1D and AITD. One of the largest family studies of T1D and AITD in the US [6,7], showed that among female diabetic probands Hashimoto's thyroiditis (HT) was diagnosed in 54–75% of cases, and among female relatives, the frequency of HT was 22–44%. Two other studies, one from the UK [8] and one from Colombia [9] showed similar results. Thus, epidemiological data support a significant shared genetic susceptibility to T1D and AITD. However, while much has been learned about the genetics of T1D and AITD individually, less is known about the joint genetic etiology of these two diseases.

In view of the strong evidence for shared susceptibility for T1D and AITD we have previously mapped joint susceptibility genes for T1D and AITD using linkage studies in a large cohort of multiplex families in which T1D and AITD clustered. We used both the candidate gene approach [10,11], and whole genome linkage approach [12]. A striking finding of these studies was that the phenotype of T1D + AITD in the same individual (APS3v) was a unique phenotype with a genetic predisposition distinct from that of T1D or AITD alone [10,12]. The most significant contribution to T1D + AITD (APS3v) genetic susceptibility came from a sequence variant in HLA-DR [13]. In addition to the HLA class II susceptibility contribution we mapped three non-MHC loci showing evidence for linkage - CTLA-4, PTPN22, and FOXP3 [10–12]. These genes as well as other genes, such as IL-2α/CD25 and TNFα have been reported by other groups studying APS3v [14]. The aim of the present study was to identify genes unique for APS3v using the robust genome wide association study (GWAS) approach in order to identify novel shared mechanisms and pathways for T1D and AITD.

2. Participants and methods

2.1. Study participants

The project was approved by the Icahn School of Medicine Institutional Review Board. SEARCH for Diabetes in the Youth study participants ≥18 years old or a parent/guardian of participants <18 years provided written informed consent for data collection including DNA. We performed a two-stage GWAS using a discovery set and an independent replication set.

Discovery set (Table 1): The discovery set included 346 non-Hispanic White (NHW) patients with T1D who were also confirmed to be positive for TPO antibodies. These patients represent a subset of the SEARCH for Diabetes in Youth study prevalent and incident cohorts (for a full description of the SEARCH cohort see Ref. [15]). T1D was based on physicians' diagnosis of T1D and confirmed at the time of the SEARCH study visit based on a positive autoantibody titer for GAD65 or IA-2, consistent with the ADA criteria for type 1A (autoimmune) diabetes [16]. Controls included 727 healthy Caucasians (NHW) that were obtained from the Illumina iControlDB database (http://genomeinformaticsalliance.org/science/icontroldb.ilmn) [17]. Patients and controls were gender and ethnicity matched. The breakdown of the discovery set recruitment is detailed in Supplemental Table G.

Replication set (Table 1 B): The replication set included a separately enrolled dataset of 185 Caucasian (NHW) patients with T1D + AITD (consisting of a T1D + AITD cohort previously described [13] with additional patients enrolled since 2010); 340 healthy Caucasians (NHW) were used as replication controls. The

Table 1A
Discovery set: non-hispanic white participants with type 1 diabetes (n = 346) from the search for diabetes in youth study, clinical characteristics and family history of diabetes.

	All	Females	Males	*p*-Value[a]
	n = 346	n = 216 (62%)	n = 130 (38%)	
Age at diagnosis (y) [Mean (SD)]	8.5 (4.2)	8.5 (3.9)	8.4 (4.7)	0.78
Age at study visit (y) [Mean (SD)]	13.9 (4.0)	13.8 (4.1)	14.1 (4.0)	0.66
Disease duration (y) [Mean (SD)]	5.0 (4.5)	4.9 (4.5)	5.1 (4.4)	0.43
TPO-Ab (IU/L) [Mean (SD)]	15.9 (10.0)	16.0 (10.1)	15.6 (9.3)	0.75
Additional diagnosis [n (%)]				
Hypothyroidism	55/346 (16%)	39/216 (18%)	16/129 (12%)	0.18
Hyperthyroidism	24/345 (7%)	17/216 (8%)	7/129 (5%)	0.51
Vitiligo	6/343 (1.7%)	3/215 (1.4%)	3/128 (2.3%)	0.68
Addison disease	1/345 (0.3%)	1/216 (0.5%)	0	1
Celiac disease	0	0	0	–
Family history of DM [n (%)]				
Mother	21/344 (6%)	9/215 (4%)	12/129 (9%)	0.065
Father	20/340 (6%)	12/214 (6%)	8/126 (6%)	1.00
Maternal grandfather	52/324 (16%)	33/198 (17%)	18/128 (14%)	0.64
Maternal grandmother	58/335 (17%)	34/208 (16%)	24/127 (19%)	0.40
Paternal grandfather	55/318 (17%)	33/199 (17%)	22/119 (18%)	0.76
Paternal grandmother	53/318 (16%)	29/207 (14%)	24/121 (20%)	0.21
Age at the time of diagnosis DM (y) [Mean (SD)]				
Mother	24.7 (11.8)	25.3 (11.2)	24.4 (12.5)	0.99
Father	31.7 (15.9)	31.5 (15.3)	32.0 (18.1)	0.95
Maternal grandfather	55.1 (15.8)	57.5 (13.5)	51.0 (18.8)	0.55
Maternal grandmother	54.6 (14.3)	52.5 (16.5)	58.9 (6.0)	0.78
Paternal grandfather	56.2 (16.5)	57.9 (16.0)	54.3 (17.5)	0.44
Paternal grandmother	51.0 (21.6)	52.4 (23.0)	49.4 (20.5)	0.52

n, number, y, years, SD, standard deviation. TPO-Ab, thyroid peroxydase antibodies, DM, diabetes mellitus (any type).
[a] *p*-Values are for comparing females vs. males.

Please cite this article in press as: Y. Tomer, et al., Genome wide identification of new genes and pathways in patients with both autoimmune thyroiditis and type 1 diabetes, Journal of Autoimmunity (2015), http://dx.doi.org/10.1016/j.jaut.2015.03.006